In re Application of: Chaim GILON et al.
Serial No.: 10/508,959
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Examiner: Desai, Anand U.
Group Art Unit: 1656
Attorney Docket: 28557

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-131 are in this Application. Claims 1-68, 79-83 and 87-131 have been withdrawn from consideration. Claims 69, 70, 73, 75, 77 and 78 have been rejected under 35 U.S.C. § 102. Claims 69-73, 75-78 and 84-86 have been rejected under 35 U.S.C. § 103. Claims 70 and 73 have been canceled herewith. Claim 69 has been amended herewith.

Amendments To The Claims

Species Election

The Examiner has stated (in Item 1 of the outstanding Office Action) that claim 74 does not read on the elected conjugate of H2A-BSA.

The Examiner has further stated (in Item 3) that no prior art was found for the elected species, H2A-BSA, and that the search has been extended to a second species that is H2A-beta galactosidase.

Applicant believes that since no prior art was found for the elected species, the claims have been examined in their generic context and hence that claim 74 currently should not be withdrawn.

Claim Objection

The Examiner has objected to claim 69 for describing a conjugate described in non-elected withdrawn claim 1. The Examiner has suggested identifying the conjugate in claim 69.

Claim 69 has been amended so as to be in an independent form, and recites, instead of *"...the conjugate of claim 1"*:

"...a conjugate comprising a histone moiety covalently linked to said macromolecule-of-interest, said histone moiety being transportable through cell membranes and importable into cell nuclei ....".

35 U.S.C. § 112 1st Paragraph Rejection

The Examiner has rejected claim 73 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The Examiner's rejection is respectfully traversed. Claim 73 has been canceled.

Specifically, the Examiner has stated that the description of any derivative of any histone protein is not described, and that the possible variations are enormous to any class of modifications. The Examiner has therefore concluded that the specification fails to provide adequate written description for the genus of the claims and does not reasonably convey to one skilled in the relevant art that the inventors had possession of the entire scope of the claimed invention.

Applicant has chosen, in order to more specifically define the structure of the conjugate recited in claim 69, to introduce the limitations recited in claim 73 into claim 69.

Consequently, claim 73 has been canceled.

Referring now to the Examiner's rejection, Applicant wishes to note that the specification describes in detail the structure of the histone moiety of embodiments of the present invention, as well as of derivatives of histone proteins suitable for use as the claimed histone moiety (see, for example, page 13, line 10 to page 14, line 13 of the instant application). Applicant therefore believes that the specification would convey to one skilled in the art that the inventors had possession of the entire scope of the claimed invention.

Notwithstanding the above, and in order to expedite prosecution, Applicant has chosen to amend claim 69 so as to recite, instead of *"derivative of a histone protein"*:

"...peptide sequence derived from a histone protein, said peptide sequence comprising a positively charged amino acid sequence and a nuclear localization signal".

Support for this amendment may be found, for example, on page 13, lines 10-13 and page 14, lines 6-9 of the instant application.

Applicant wishes to note that one skilled in the art would readily be able to determine which sequences derived from a histone protein correspond to the abovementioned positively charged amino acid sequence and nuclear localization signal.

Applicant therefore contends that the claimed histone moiety is clearly defined by the structural features of the protein and hence is adequately described.

Applicant therefore believes to have overcome the Examiner's rejection.

35 U.S.C. § 102(b) Rejection

The Examiner has rejected claims 69, 70, 73, 75, 77 and 78 under 35 U.S.C. 102 (b) as being anticipated by Baake et al. The Examiner's rejection is respectfully traversed. Claims 70 and 73 have been canceled. Claims 69 has been amended.

Specifically, the Examiner has stated that Baake et al. disclose the conjugation of histone H2A with beta-galactosidase, that membrane translocation of the conjugate was detected by fluorescence, and that the H2A has a nuclear localization peptide sequence for transport of the fusion construct. The Examiner has further stated that nuclear expression requires transport across the nuclear membrane.

Since, as argued in detail hereinbelow, Baake et al. teach a H2A-beta galactosidase conjugate which is expressed **in** cells and transported to the nucleus, Baake et al. fail to teach that the H2A-beta galactosidase conjugate is capable of transport **across the plasma membrane**.

The conjugate taught by Baake et al. is expressed in HeLa cells and transported to the nucleus of the HeLa cells (see, for example, page 338, legend of Figure 2, in

Baake et al.). Because the conjugate is never outside the cell, it is not transported into the cell through the plasma membrane. The conjugate is transported from the cytosol of the HeLa cell to the nucleus of the same cell, thereby being transported across the nuclear membrane but not across the plasma membrane. While according to the teachings of Baake et al., the nuclear localization peptide sequence (NLS) of the conjugate provides the conjugate with the ability be transported across the nuclear membrane, the presence of NLS is not expected to provide the conjugate with an ability to cross another membrane, a plasma membrane in particular.

In sharp contrast, the present inventors have surprisingly uncovered that conjugates comprising a histone moiety are useful for delivering a macromolecule-of-interest across a plasma membrane into a cell cytoplasm (see, for example, page 12, lines 23-28 of the instant application), in addition to a cell nucleopasm. Thus, for example, co-incubation of a cell and the conjugate results in delivery of the conjugate, and a macromolecule-of-interest comprised by the conjugate, into the cell.

Since the conjugate taught in Baake et al. is expressed in the cell, no membrane transportability of the conjugate is taught in this document and further, no such transportability which is effected upon co-incubation of the conjugate and the cell, is taught therein.

Furthermore, the present inventors have surprisingly uncovered that the mechanism involved in delivering a macromolecule-of-interest conjugated to a histone moiety into a cell differs from the mechanism of transport into the nucleus. Thus, the present inventors have uncovered that the mechanism of delivery into a cell with the claimed conjugates does not require active transport (i.e., is energy-independent) and is non-endocytic (i.e., does not depend on endocytosis). See, for example, page 12, lines 23-26; and page 51, line 1 to page 52, line 9 of the instant application).

Energy independence of the mechanism of delivery is demonstrated for example, by the fact that delivery into the cell is independent of ATP concentration (see for example, page 51, lines 1-11; page 53, lines 16-21; and page 55, lines 15-18

of the instant application). A non-endocytic mechanism is evident due to the demonstrated energy independence, as described hereinabove, as well as the findings that delivery into the cell is independent of temperature and is effected both at 37 °C and at 4 °C (see for example, page 49, lines 29-32; page 53, lines 7-15; page 54, lines 3-7; and page 55, lines 6-8 of the instant application), is independent of presence of inhibitors of endocytosis (see for example, page 51, line 11 to page 52, line 9; page 54, lines 7-11; and page 55, lines 3-6 of the instant application) and does not exhibit saturation effects, which are characteristic of receptor-mediated mechanisms such as endocytosis (see for example, page 49, line 32 to page 50, line 1; page 53, line 22 to page 54, line 2; and page 55, lines 18-21 of the instant application).

Hence, it is clear that the ability of the conjugates described in the instant application to deliver a macromolecule-of-interest into a cell is not based on the ability of the conjugates to be transported into a cell nucleus.

Notwithstanding the above, and in order to expedite the prosecution, Applicant has chosen to amend claim 69 so as to more clearly distinguish embodiments of the present invention from the teachings of Baake et al.

Hence, claim 69 has been amended so as recite *"co-incubating the cell and a conjugate..."* instead of *"contacting the cell with a conjugate"*.

Consequently, claim 70, which recited the limitation now added to amended claim 69, has been canceled.

As would be recognized by any person skilled in the art, the term "co-incubating" clearly indicates that both the conjugate and the cell are present in a medium that surrounds the cell and that the conjugate is originally *outside* the cell, in sharp contrast to the teachings of Baake et al.

Applicant therefore believes that amended claim 69, as well as claims 75, 77 and 78, which depend directly or indirectly therefrom, are not anticipated by Baake et al., and are therefore allowable.

35 U.S.C. § 103(a) Rejection

The Examiner has rejected claims 69-73, 75-78 and 84-86 under 35 U.S.C. 103(a) as being unpatentable over Baake et al. in view of Guo et al. The Examiner's rejection is respectfully traversed. Claims 70 and 73 have been canceled. Claim 69 has been amended.

Specifically, the Examiner has stated that Baake et al. do not explicitly disclose a method of delivering a therapeutic or non-marker macromolecule with a H2A membrane translocating peptide, and do not disclose the use of a spacer, but that Guo et al. disclose the use of a fusion protein, which may comprise a linker and/or a nuclear localization sequence, for delivery of a compound including a peptide and a protein. The Examiner has therefore concluded that it would have been obvious to one of ordinary skill in the art to substitute components, such as nuclear localization peptide sequences, with other known nuclear localization peptide sequences, to transport macromolecules-of-interest across membranes.

As argued hereinabove, Baake et al. fails to teach that conjugates comprising a histone moiety are capable of delivering a macromolecule-of-interest into a cell. It is noted that the claimed invention clearly differs from the teachings of Baake et al., regardless of the nature of the macromolecule-of-interest and of the linker between the histone moiety and macromolecule-of-interest.

Guo et al. teach membrane penetrating peptides (MPPs) capable of penetrating cells, which are useful for delivery of compounds of interest to cells. Guo et al. do not teach MPPs comprising a histone moiety.

It is therefore clear that one skilled in the art would have no motivation to combine the membrane penetrating peptides of Guo et al., which do not comprise a histone moiety, with the conjugate of Baake et al., which comprises a histone moiety, but is not taught as being capable of penetrating cells, since none of these documents, nor any other art, provides any evidence for the capability of a histone moiety or a

histone moiety covalently linked to a macromolecule to transport through a cell plasma membrane.

Applicant wishes to note that although the MPPs taught by Guo et al. are based on a nuclear localization sequence (NLS), Guo et al. teach that this NLS differs from previously identified NLSs (see, for example, paragraph [0042] of Guo et al.). As is well known in the art, NLSs are characteristic of nuclear proteins in general, and many different types of NLSs exist. Hence one skilled in the art would have no expectation that replacing the NLS taught by Guo et al. with a different NLS, such as the NLS taught by Baake et al. as being present in H2A, would result in a conjugate capable of delivering a macromolecule-of-interest into a cell. Moreover, one skilled in the art would have no expectation that the conjugates described in the instant application are capable of delivering a macromolecule by an energy-independent, non-endocytic mechanism.

Applicant therefore believes that amended claim 69, as well as claims 71 and 72, 75-78 and 84-86, which depend directly or indirectly thereupon, are not rendered obvious by Baake et al. in view of Guo et al., and are therefore allowable.

In view of the above amendments and remarks it is respectfully submitted that amended claim 69, claims 71 and 72, 74-78 and 84-86 are now in condition for allowance. A prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

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Encls.
Petition for Extension for three (3) months time
Amendment Claim Transmittal